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Request for grant of a patent

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1.	Your reference	CDK2112
2.	Patent application number *(The Patent Office will fill in this part)*	0314365.8
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	RHODIA CONSUMER SPECIALTIES LIMITED Oak House Reeds Crescent Watford Hertfordshire, WD24 4QP.
		20JUN03 E816504-8 002806 P01/7700 0.00-0314365.8
	Patents ADP number *(if you know it)*	7870322006
	If the applicant is a corporate body, give the country/state of its incorporation	England
4.	Title of the invention	UNCOUPLING AGENT
5.	Name of your agent *(if you have one)*	Barker Brettell
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	138 Hagley Road Edgbaston Birmingham B16 9PW
	Patents ADP number *(if you know it)*	7442494002
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country / Priority application number *(if you know it)* / Date of Filing *(day/month/year)*
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application / Date of filing *(day/month/year)*
8.	Is a statement of inventorship and of right to grant of a patent required in support of this request *(Answer 'Yes' if: a) any applicant named in part 3 is not an inventor, or b) there is an inventor who is not named as an applicant, or c) any named applicant is a corporate body. See note (d))*	YES

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Description	18 + 18
Claim*(s)*	-
Abstract	-
Drawing*(s)*	-

10. If you are also filing any of the following, state how many against each item.

Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
Request for preliminary examination *(Patents Form 9/77)*	-
Request for substantive examination *(Patents Form 10/77)*	-
Any other documents *(please specify)*	-

11. I/We request the grant of a patent on the basis of this application.

Signature Barker Brettell Date

Barker Brettell 19 June 2003

12. Name and daytime telephone number of person to contact in the United Kingdom — Colin D. Kinton — Tel: 0121 456 1364

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UNCOUPLING AGENT

This invention relates to a novel uncoupling agent for use in the control of bacterial biomass in aqueous systems, to the use of such an agent and to a method of using such an agent.

Bacterial biomass produced during the treatment of wastewater is costly to dispose of. Hitherto, organic chemical compounds have been used as "uncoupling agents" to reduce bacterial biomass by uncoupling a proton gradient across the plasma membrane of bacteria. The proton gradient is used *inter alia* promote the uptake of nutrients from the environment and to generate ATP (adenosine triphosphate) via oxidative phosphorylation. Uncoupling of the proton gradient causes reduced ATP synthesis. Reduced ATP synthesis results in a decrease in biomass.

However, the aforementioned uncoupling agents are usually phenolic or halogenated products and are unsuitable for use in aqueous systems due to high toxicity.

The present invention provides a novel uncoupling agent with low toxicity to aqueous systems.

Accordingly, the present invention, in a first aspect, provides an uncoupling agent comprising an alkyl-substituted phosphonium compound of formula (I).

$$[\ (R)_4\ P^+]_n\ X$$

wherein

n is the valency of X,

R is an alkyl moiety and X is an anion.

Suitably, R is an hydroxyalkyl group. Preferably, R is an hydroxymethyl group.

X is preferably selected from the group consisting of chloride, sulphate and bromide.

The alkyl-substituted phosphonium compound is preferably tetrakis (hydroxymethyl) phosphonium sulphate. Alternatively, the alkyl-substituted phosphonium compound is selected from tetrakis (hydroxymethyl) phosphonium chloride and tetrakis (hydroxymethyl) phosphonium bromide.

The alkyl-substituted phosphonium compound according to the first aspect of the present invention may be formulated with at least one of the following:

a surfactant;
a scale inhibitor;
a corrosion inhibitor;
a biocide; and
a dispersant.

The present invention also provides, in a second aspect, the use of an effective amount of an uncoupling agent as defined in the first aspect of the present invention to control bacterial biomass in an aqueous system.

Preferably, the aqueous system is an oil field. Alternatively, the aqueous system may be a water treatment plant, a system used in paper production or......

The effective amount of said uncoupling agent is preferably between x and y.

The present invention further provides, in a third aspect, a method for controlling bacterial biomass in an aqueous system comprising adding to, or contacting with, said aqueous system an effective amount of an uncoupling agent according to the first aspect of the invention.

The effective amount of the uncoupling agent is preferably between x and y.

The invention will now be described with reference to the following example.

Example 1

Materials and Methods

A laboratory test was developed to determine the impact of chemical compounds on respiration parameters through a complete oxygen consumption pattern analysis using the Monod's model.

Growth medium:

Glucose:	500 mg/l
Yeast extract Difco:	50 mg/l

Mineral nutrients: as described in Standard Method ISO 9888 (Determination of ultimate aerobic biodegradability in aquatic environment)

Medium seeding: 50 mg/l homogenized and washed sample from municipal activated sludge treatment unit.

Incubation: 7 days at 20 °C in BOD-meter bottles OxiTop Control (WTW, D-82362 Weilheim).

The oxygen consumption was automatically determined and recorded all through the growth and decay phases (360 data/assay).

Data was interpreted using a simple growth mathematical model described in the appendix.

The determination of the model's coefficients are made through adjustment using the statistical test χ^2 to fit model to the experimental data.

$$\chi^2 = \sum \frac{[OC_{observed} - OC_{theoretical}]^2}{OC_{theoretical}}$$

where OC is the oxygen consumption

Results

Biocides TOLCIDE ® PS75 and TOLCIDE ® PS50 were tested at the following concentration:

0 (Control culture) ; 1 ; 2.5 ; 5 ; 10 and 25 μl/L

Each set of experiments was completed with 20 mg/L 2,4-DNP culture

Oxygen consumption curves are presented in appendix 2

Calibrated curves of the model to the experimental data are presented in appendix 3

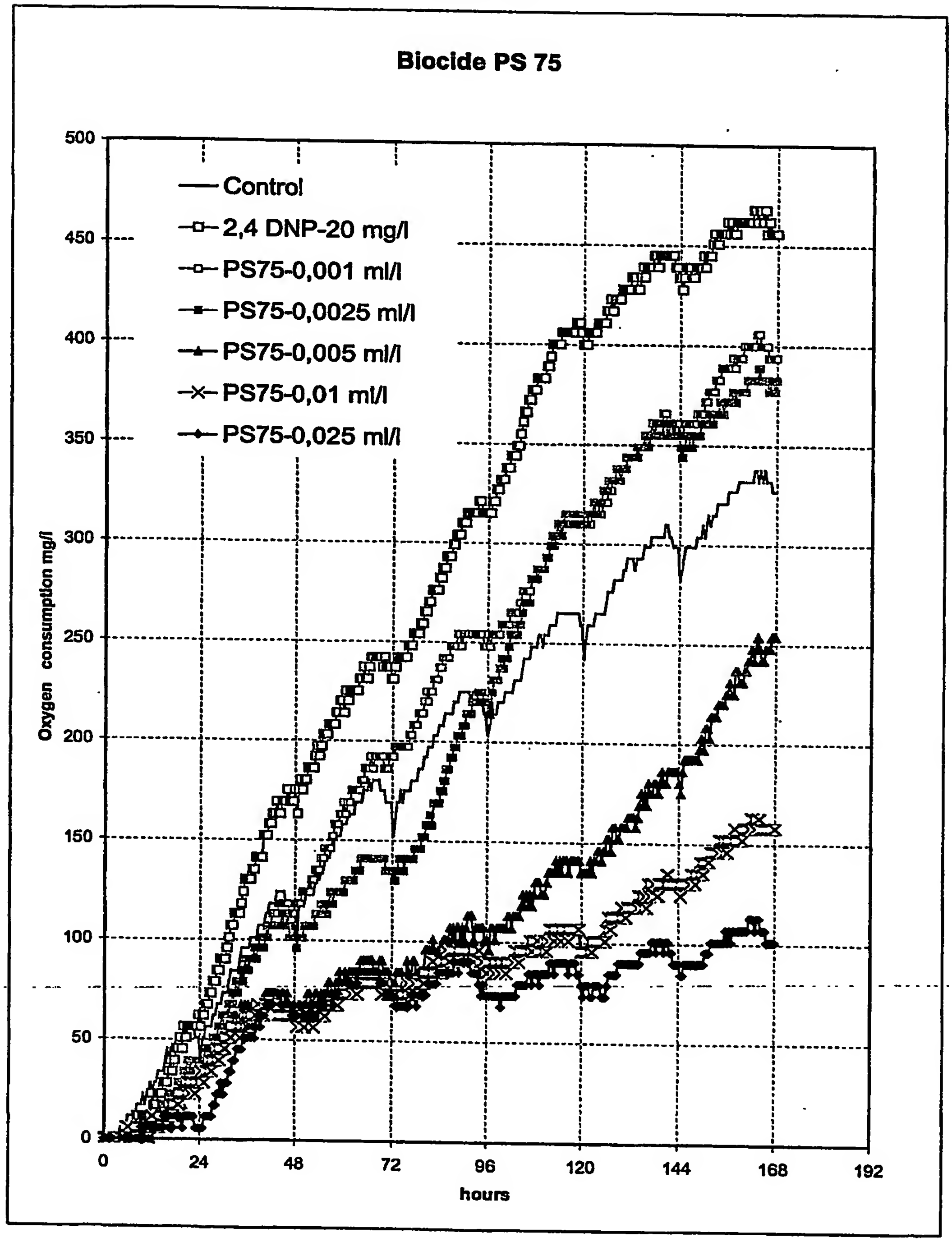
The table below presents the calculated growth coefficients values best fitting to experimental data

	a g biomass / g substrate	b g biomass/g biomass.d	k g substrate/g biomass.d
Control	0.55	0.09	2.5
2,4 DNP 20 mg/l	0.28	0.18	2.5
PS50 1 μl/L	0.48	0.1	2.43
PS50 2.5 μl/L	0.35	0.14	2.33
PS50 5 μl/L	0.34	0.15	2.40
PS50 10 μl/L	0.36	0.14	1.38
PS50 25 μl/L	0.22	0.23	0 .75
PS75 1 μl/L	0.41	0.12	2.25
PS75 2.5 μl/L	0.37	0.13	1.93
PS75 5 μl/L	0.36	0.14	1.13
PS75 10 μl/L	0.33	0.15	0.80
PS75 25 μl/L	0.17	0.30	0.63

The biocides TOLCIDE PS50 and TOLCIDE PS75 exhibit both uncoupling and inhibition effects on mixed bacterial consortium growth. Optimal uncoupling effect are obtained at 1 μl/L and 5 μl/L for TOLCIDE PS75 and TOLCIDE PS50 respectively.

These biocides could thus be employed to reduce biological sludge production during wastewater treatment.

Impact of TOLCIDE PS75 on oxygen consumption kinetics



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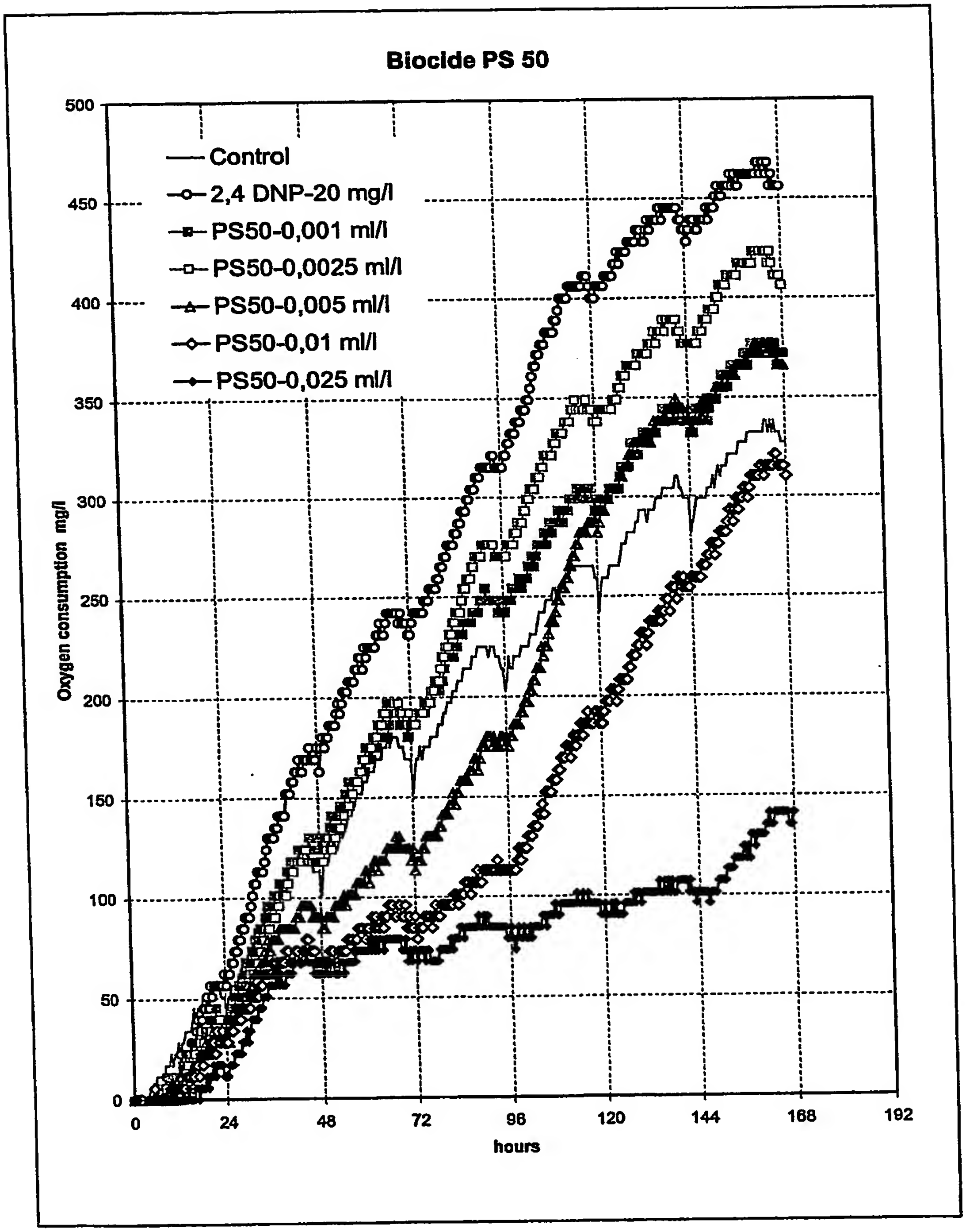
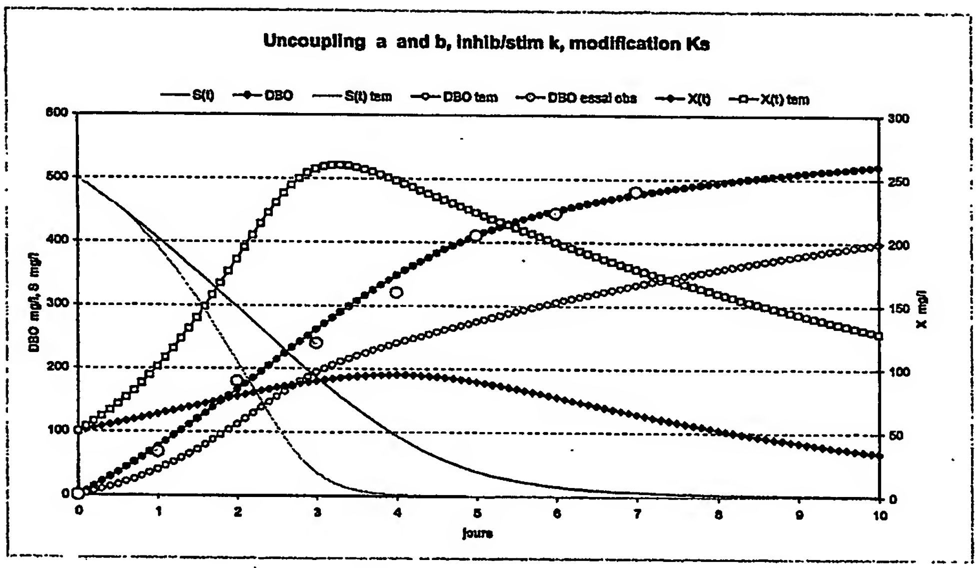
Impact of TOLCIDE PS50 on oxygen consumption kinetics



Biocide PS50

a:	2,4-DNP	20 mg/L	
b:	PS50	1	µl/L
c	PS50	2,5	µl/L
d	PS50	5	µl/L
e	PS50	10	µl/L
f	PS50	25	µl/L

a



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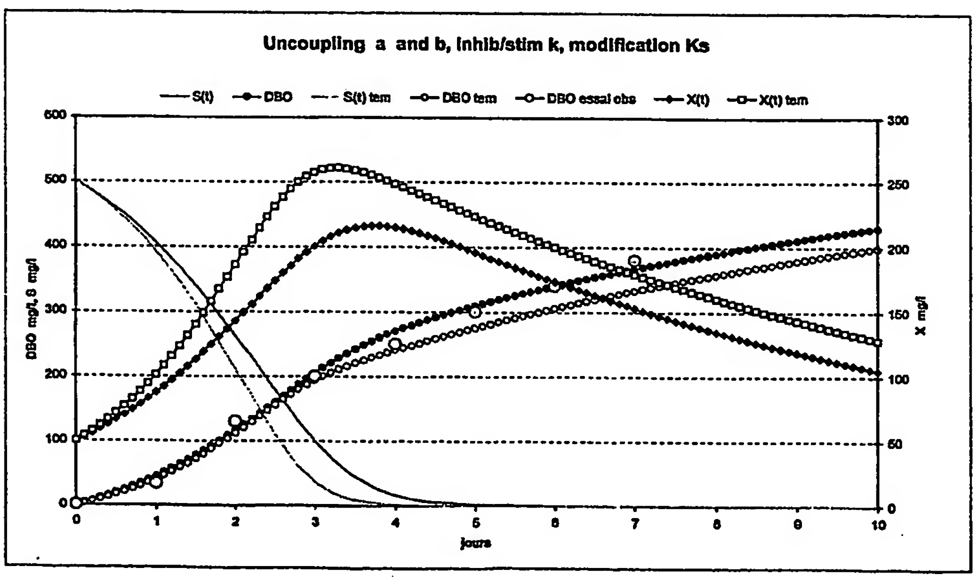

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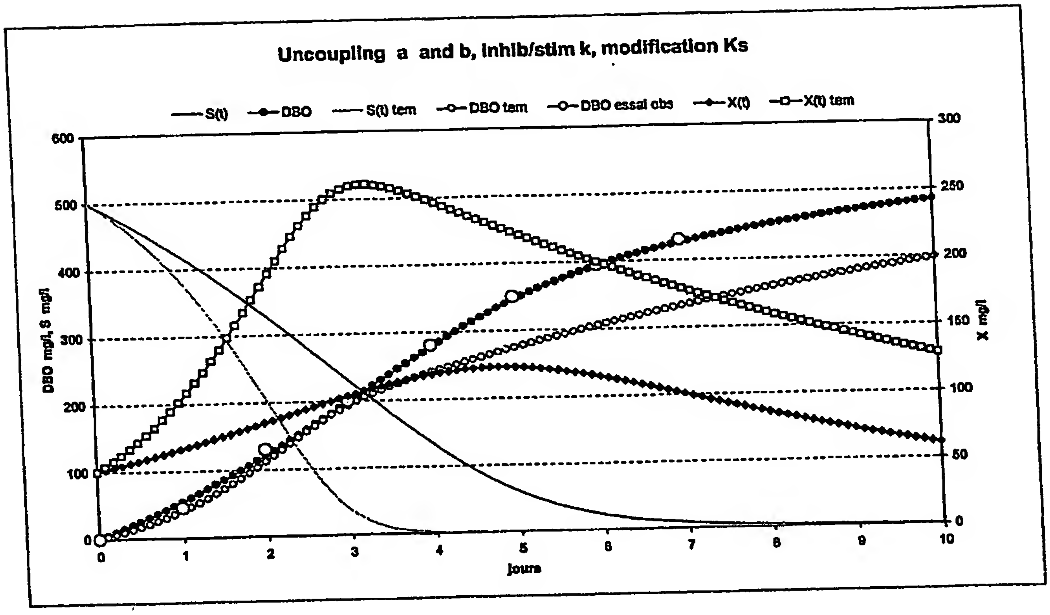

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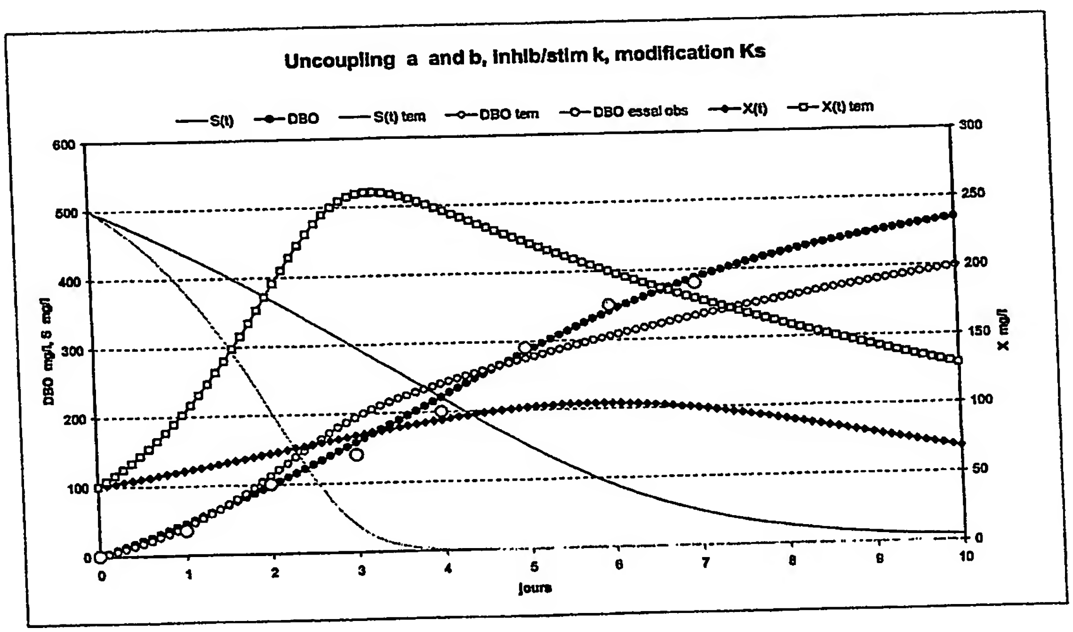

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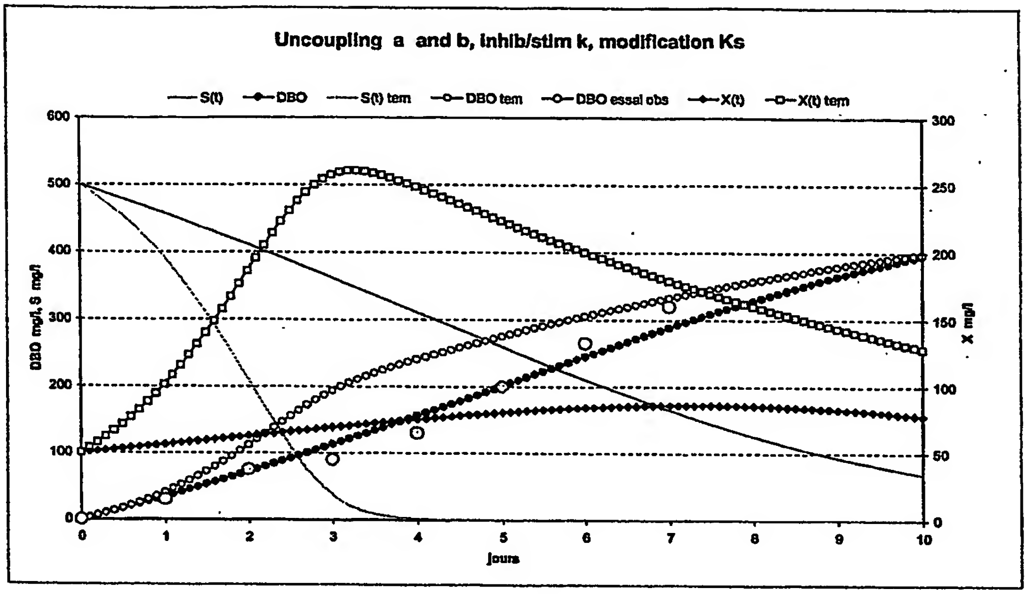

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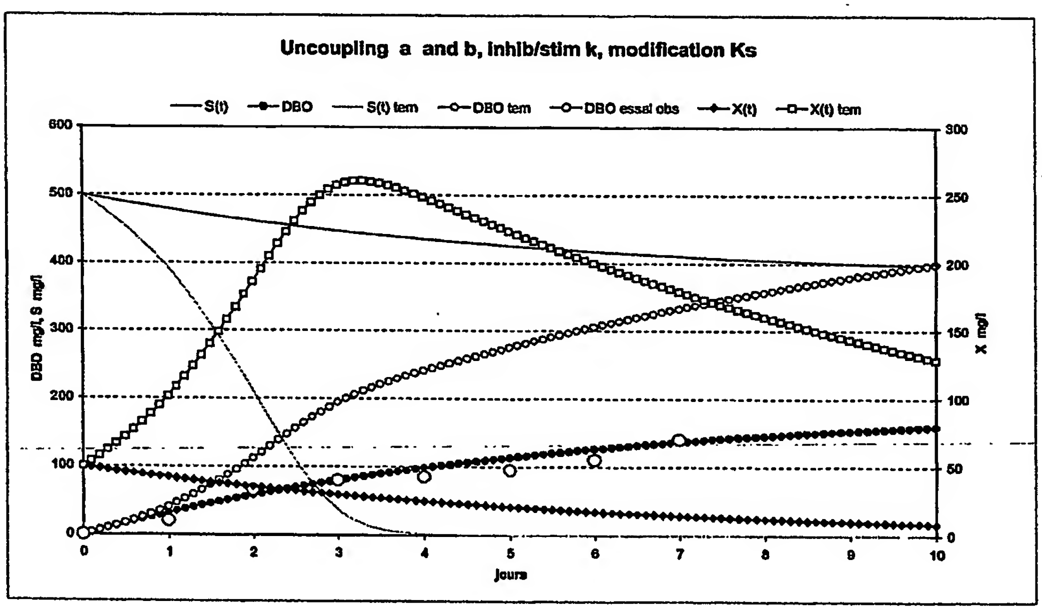

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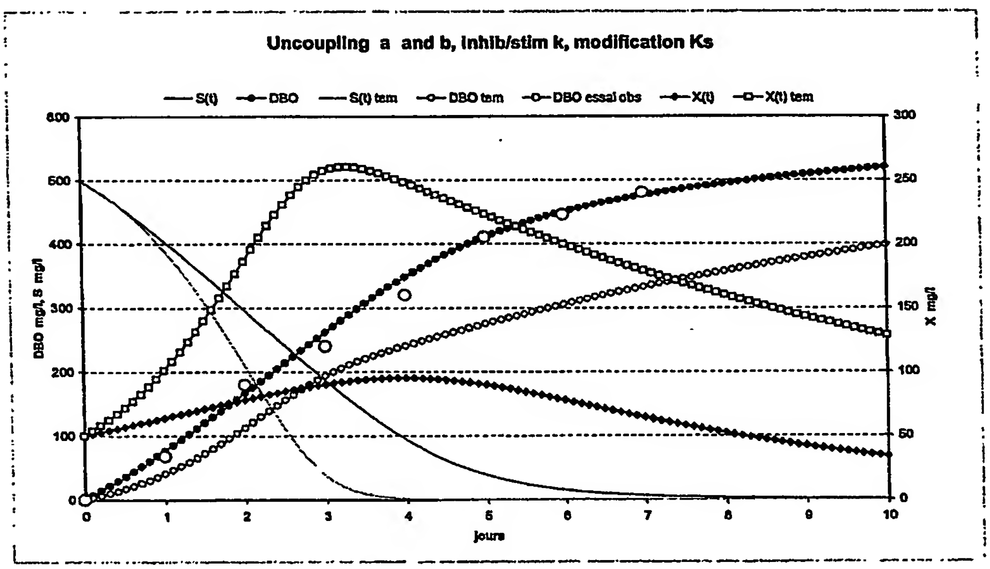
Jours

Biocide PS75

a:	2,4-DNP	20	mg/L
b:	PS75	1	µl/L
c	PS75	2,5	µl/L
d	PS75	5	µl/L
e	PS75	10	µl/L
f	PS75	25	µl/L

a



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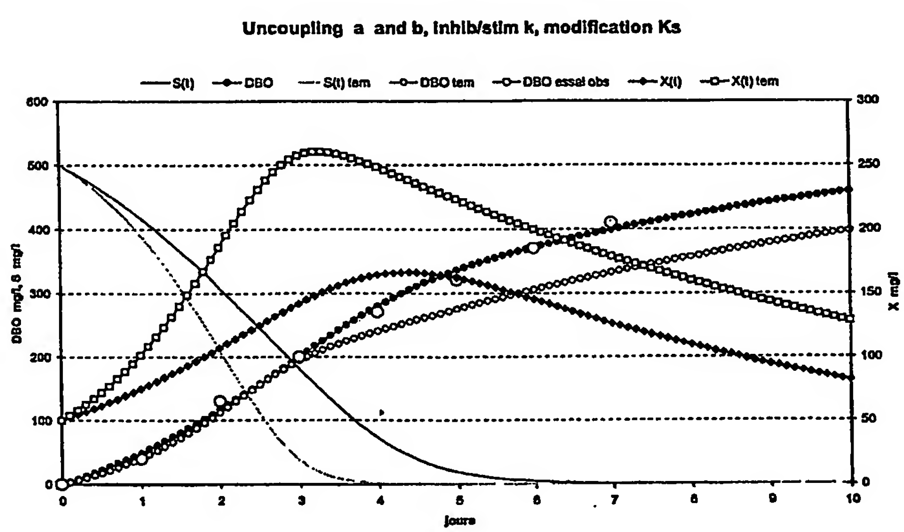

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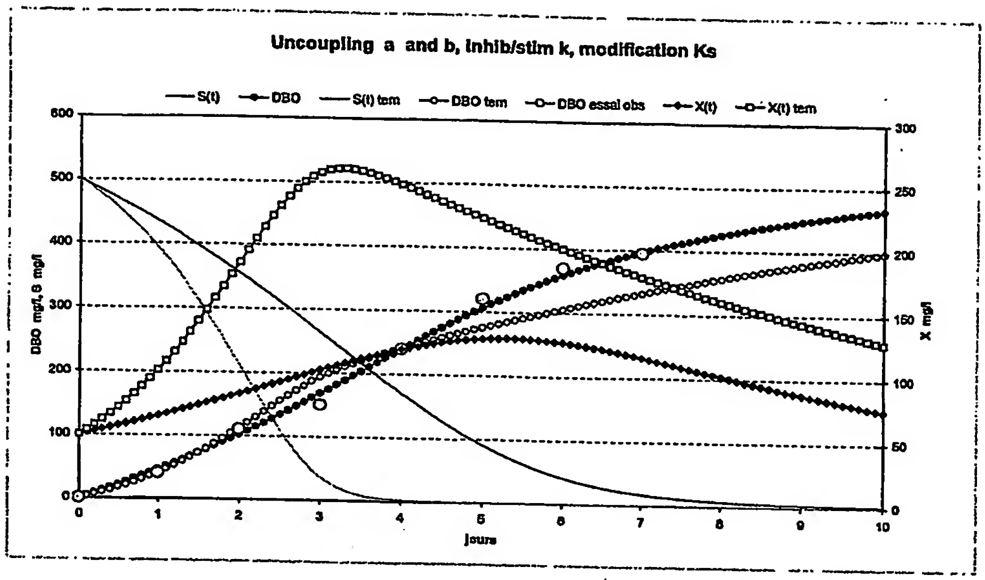
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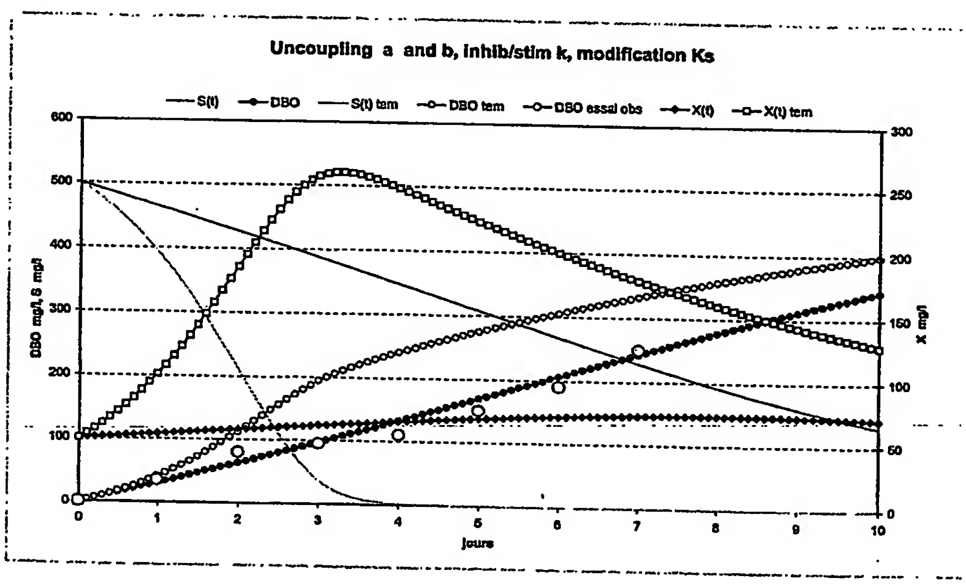
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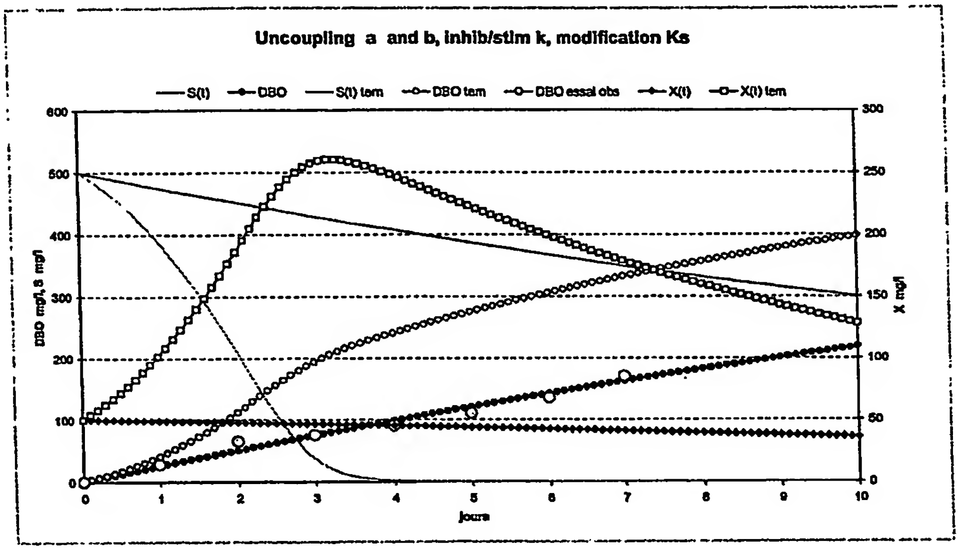
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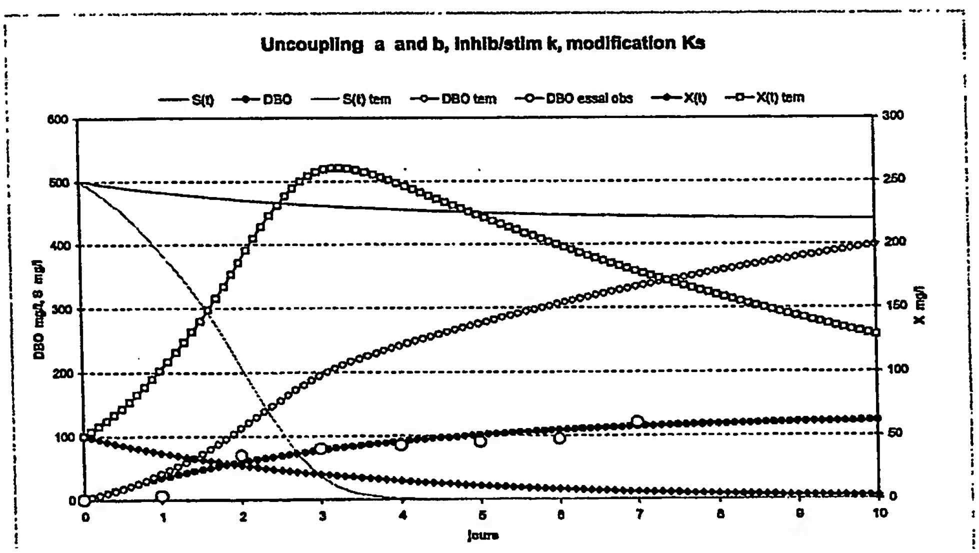
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Appendix

Bacterial growth model

$$\frac{d[X]}{dt} = a \cdot \frac{d[S]}{dt} - b \cdot [X]$$

$$\frac{d[S]}{dt} = k \cdot [X] \cdot \frac{[S]}{K_S + [S]}$$

$$\frac{d[O_2]}{dt} = a' \cdot \frac{d[S]}{dt} + b' \cdot [X]$$

[X] : Concentration in biomass amount subject to endogenous metabolism g MLSS/L

d[X]/dt : Biomass growth rate , g dry biomass/L.d

d[S]/dt : Carbon substrate consumption rate, g /L.d

a : Intrinsic substrate-biomass growth yield, g X/g substrate consumed

b : Biomass maintenance coefficient, g dry biomass/g dry biomass. d

$d[O_2]$ /dt : Oxygen consumption rate, g O_2/L.d

a' : Specific oxygen consumption coefficient, g O_2/g organic substrate consumed.

b' : Endogenous respiration coefficient, g O_2/g X

k : Maximum specific activity, g S/ g dry biomass.d

K_s : Apparent substrate affinity coefficient or half maximum specific activity substrate concentration, mg/l

Relationship between a and a' : a' + (b'/b) * a = 1

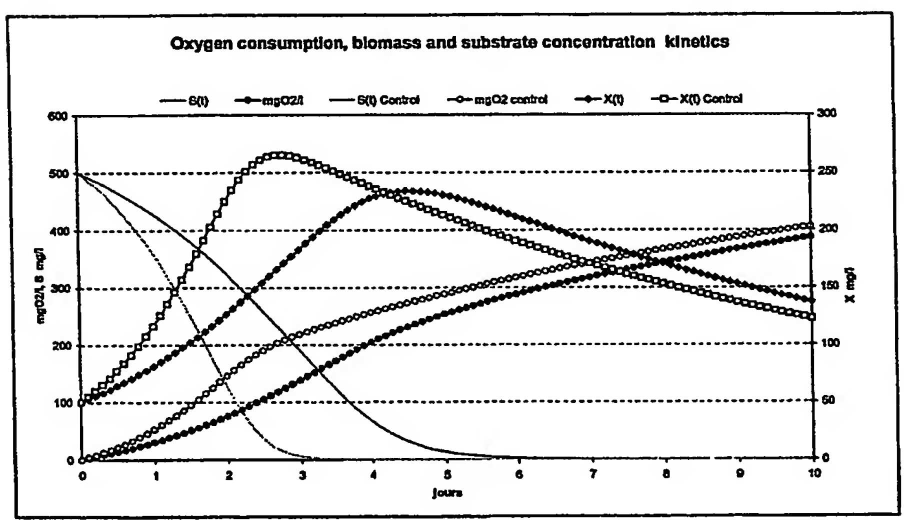
Relationship between b and b' : b' = 1.25 * b

Computation of this mathematical model generates oxygen consumption patterns typical of growth inhibition, stimulation, uncoupling and a combination of inhibition/stimulation - uncoupling which are shown by the graphs below.

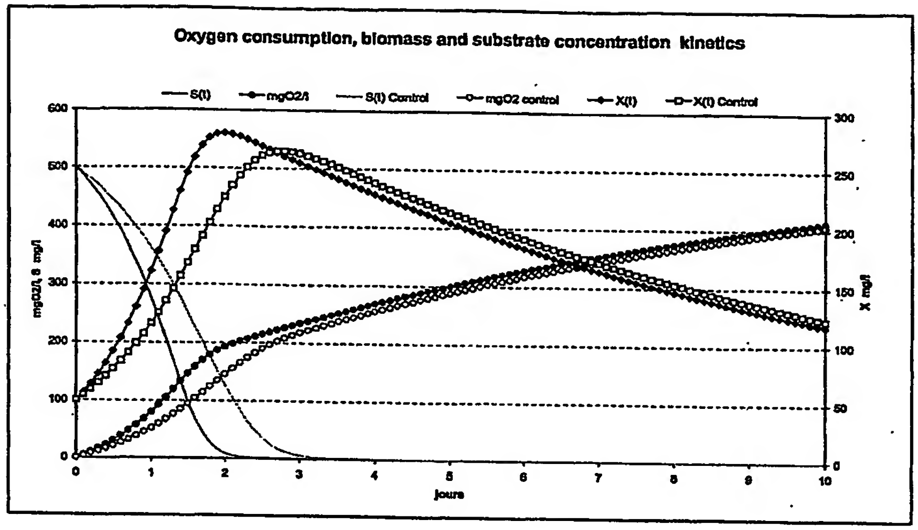
Typical oxygen consumption patterns

Control
Growth Inhibition (maximum specific activity augmented)
Growth stimulation (maximum specific activity reduced)
Uncoupled growth
Uncoupled and stimulated growth
Uncoupled and inhibited growth

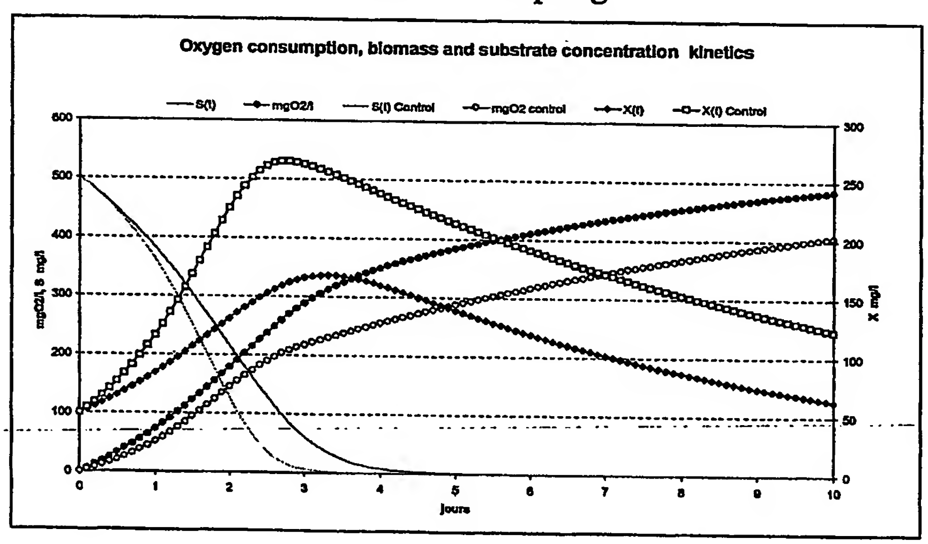
Growth inhibition



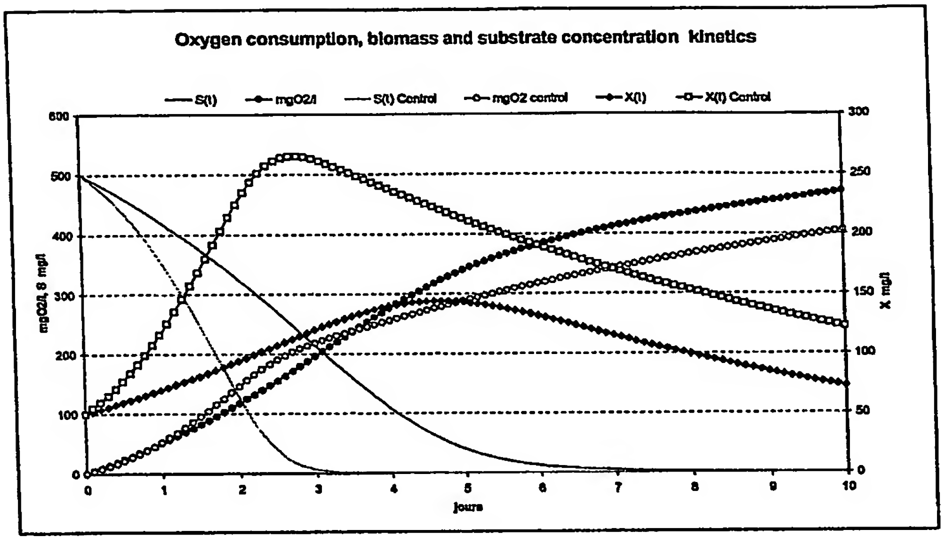
Growth stimulation



Growth uncoupling



Growth uncoupling and inhibition



5 ## Growth uncoupling and stimulation